USDA-ARS Vegetable and Forage Crops Research Unit, Prosser, WA

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Potatoes

(Ashok Alva, Rick Boydston, Chuck Brown, Hal Collins, Jim Crosslin, Roy Navarre)

1. Developing high-phytonutrient potatoes.

Recent surveys found that consumer's often perceive that potatoes lack good nutrition and are not known for their health benefits. This perception is thought to be a primary factor in the decline of potato consumption that is threatening the sustainability of the US potato industry. We continue investigations towards developing high-phytonutrient potatoes using multiple independent approaches. We determined that developmentally young tubers contain higher amounts of some phytonutrients than those in fully mature tubers. In a 2009 field trial, we grew 90 genotypes, then screened them for phytonutrient content using LCMS. Among this germplasm we identified several genotypes that had good yields of small tubers (essential for profitability), good appearance, taste and had antioxidant amounts that rival vegetables such as spinach, broccoli and even kale. We recently constructed several vectors that will allow us to overexpress or silence targeted potato genes in a tuber specific manner and have cloned various potato genes involved in folate, anthocyanin, protein or phenolic biosynthesis. Engineered plants are being constructed to see if we can generate potatoes with even higher amounts of these phytonutrients. High-phytonutrient "baby potatoes" could help restore the healthy image of potatoes, increase demand and provide ample choices for consumers.

2. Development of molecular markers for the prediction of super high carotenoids in potato genotypes.

Potato is a good source of dietary lutein and zexanthin, components of the human retina. Breeding for higher levels of these components of the tuber flesh has been difficult because we have not known what gene alleles need to be assembled. We have found that the presence of two markers *BCH-2* and *zep* are necessary for the expression of high levels of carotenoids. Now we have a test that can be applied to new potato genotypes when they are tiny plants. The value of high carotenoid potatoes is that the higher levels of lutein and zeaxanthin make the potato a superior food that promotes eye health. Now that we can predict carotenoid expression we will be able to produce varieties that have high yield and super-high carotenoids.

3. First report of new variant of cherry leaf roll virus in potato.

Many viruses infect potatoes and plants closely related to potatoes. Some of these potato relatives possess characteristics that can be useful in potato breeding programs, such as resistance to frost, pests, or diseases. Because of these desirable genetic characteristics, some of these plants, including "wild potato", Solanum acaule, have been used in potato breeding programs. About two years ago a virus was detected by personnel at USDA-APHIS that had been introduced into the United States in true seed of Solanum acaule. Because the virus was transmitted through the seed it posed a potential threat to progeny potatoes that might have had this material as a parent. Early work by the plant pathologists failed to identify this new pathogen. Plant material infected with the virus, designated JCM-79, was obtained under APHIS permit and the virus was maintained under quarantine at the USDA-ARS, Prosser, location. Over the course of the next few months, the virus was identified as a new variant of cherry leaf roll virus (CLRV), never previously reported in potatoes or potato relatives. Methods of detecting and identifying the new virus were developed. Identification of the virus alerted personnel involved in potato breeding programs around the world that the virus posed a threat to these programs. Development of methods for the rapid, sensitive, and specific detection and identification of the virus provides a means for potato breeding programs to test for this new pathogen in their breeding materials.

4. Deficit Irrigation for Potatoes in the Pacific Northwest.

Potato is very sensitive to water stress, which results in significant reductions in tuber yield and quality. The severity of response depends on: crop growth stage, as well as the degree of water deficit. In the dry, low-rainfall (<200 mm/year) region in Columbia Basin along eastern Washington and Oregon, the current practice is to irrigate potatoes to replenish full evapotranspiration (ET). As a result, up to 800 mm of water is applied during the growing season in this region. Increasing and rather competitive pressures for available water resources necessitate exploring water-saving techniques without sacrificing yield and/or quality, thus, the need for this study. Our study, conducted in Columbia Basin region in eastern Washington, using two cultivars ('Ranger Russet' and 'Umatilla Russet'), revealed that deficit irrigation, i.e. to replenish 80% of evapotranspiration (ET) resulted in 7 to 10% yield reduction as compared to that with full ET irrigation. However, yield reduction was up to 24% with irrigation to replenish 70% of ET. The tuber yield reduction in deficit irrigation was mainly associated with reduction in large size tubers, i.e. >0.227 kg/tuber size. Tuber specific gravity also decreased significantly with deficit irrigation as compared to that with full irrigation. Therefore, the adaptation of deficit irrigation for potatoes is largely dependent on the competing pressure for available water, economics of yield losses vs. cost of irrigation, and environmental concerns with respect to leaching of water and nutrients.

Edible Legumes

(Richard Larsen, Phil Miklas, Lyndon Porter)

1. Identification and management of metalaxyl resistant *Pythium ultimum* that is responsible for seed rot of vegetables and potato

Growers in the Columbia Basin of Washington and Oregon are experiencing a reduction in germination of pea and other vegetable crops grown under center pivot irrigation. In addition, an

unusually aggressive strain of Pythium leak on potatoes was identified in Oregon. The primary pathogen associated with vegetable seed rot and Pythium leak on potato is *Pythium ultimum*. Vegetable seed rot and Pythium leak have been managed by growers through the application of the systemic fungicide metalaxyl. Due to the increased seed and tuber rot despite treated with metalaxyl, suspicions of the development of metalaxyl-resistant isolates of *P. ultimum* were investigated. We identified the widespread presence of metalaxyl-resistant isolates of *Pythium ultimum* in growers fields in WA, OR, and ID. Nearly one out of every three fields in the Columbia Basin of Washington had metalaxyl-resistant isolates of *P. ultimum* and that these isolates were 25-fold more resistant to metalaxyl than the sensitive isolates. The resistant isolates were more aggressive than the sensitive isolates based on growth rates and that cyazofamid is the only fungicide that is affective against the resistant isolates. Growers were informed of fields with and without metalaxyl-resistant Pythium so they could make better management decisions on whether to continue using metalaxyl in their disease management programs in certain fields, saving them time and money.

2. Developing resistance to common bacterial blight in dry beans without negative effects on yield.

Common bacterial blight is a seed-borne disease that limits dry bean production worldwide. Genetic resistance can be used to control this disease, but the most effective resistance genes derive from exotic dry bean sources. When these genes from exotic sources are transferred to commercial cultivars they may reduce the yield as compared to that of the cultivar without this source of resistance, particularly when grown in disease free conditions. We transferred two exotically-derived genes into adopted dry bean lines. Extensive yield testing of these lines across three locations and two years indicated that the genes could be deployed without harming yield potential in environments which lacked disease. The same genes provided a 20% yield increase under severe bacterial disease pressure. Our finding gives dry bean breeders the green-light to widely deploy these resistance genes to effectively combat common bacterial blight disease worldwide. The yield savings from deployment of these genes across regions with high disease levels will be in the millions of dollars on an annual basis.

3. Developing resistance to pea enation mosaic virus in peas.

Pea enation mosaic virus (PEMV) is a serious disease of fresh market and dry pea in the Pacific Northwest region of the U.S. The dominant *En* gene confers resistance to PEMV in pea, however, only a limited number of available cultivars contain the gene. In addition, the gene is known to cause a yield drag in some cultivars. While some cultivars have been reported with partial resistance to PEMV, no sources of true tolerance to the virus have been reported. Four advanced pea breeding lines that do not contain the *En* gene were identified that demonstrated tolerance to PEMV as indicated by pronounced symptoms typical of PEMV infection including vein clearing and translucent flecks on leaves, and bumps or outgrowths on pods. The four pea lines evaluated did not exhibit a recovery response to infection as observed by the expression of the severe symptoms, but rather, growth rate, pod and seed yield and seed quality were not significantly affected by the virus during any growth stage of the plant. Three of the four lines were shown to lack significant differences in virus concentration compared to that of the susceptible controls, which indicates that virus concentration did not affect plant growth or seed yield. The virus concentration of the

fourth pea line, however, was significantly less than the controls and is the result of a resistance response very unique to this line. The identification of these pea lines highly tolerant to PEMV provides breeders and growers new and unique sources of tolerance when incorporation of the *En* resistance gene may not be possible or desirable. The presence of one or more quantitative trait loci (QTL) may explain the tolerance response to PEMV. Additional research will be focused on identification of these QTL and their relationship, if any, with the *En* gene in pea.

Forage Crops

(Rick Boydston, Richard Larsen, Lyndon Porter)

1. Marker development for resistance to lodging in alfalfa.

Resistance to lodging is a critical trait for alfalfa because lodging results in reduced forage yield and quality, along with decreased seed yields. The influence of environmental factors such as wind, rain, and overhead irrigation make it difficult to screen alfalfa for lodging resistance. Molecular markers associated with lodging resistance are being identified that can be used in selecting alfalfa plants for resistance to this trait. Knowledge will be gained on the inheritance of lodging resistance in alfalfa to provide breeders with tools for more rapid and precise selection of resistant plants.

2. Markers specific to race 1 and race 2 of the soilborne alfalfa root rot pathogen *Aphanomyces* euteiches.

Aphanomyces euteiches occurs in many regions of the U.S. as well as Canada, Europe and Australia. Currently Race 1 and Race two of the pathogen have been identified. Race 2 is highly significant because it overcomes commercially available sources of Race 1 resistance in alfalfa. The two physiological races currently can be differentiated only by host response on alfalfa. Molecular markers will be identified that can be used for rapid diagnosis of *A. euteiches* Race 1 and Race 2 in plant tissue and soil samples. With development of these markers, identification of Race 1 or 2 can be conducted in as little as one day, thus eliminating cumbersome and time-consuming tests in a greenhouse. Several cultures of both races have been isolated and DNA is being extracted for use in the marker screening.

3. Selection for resistance to alfalfa root rot.

Alfalfa breeders are actively attempting to develop varieties with resistance to race 2 of *Aphanomyces euteiches* because the great majority of currently available varieties are susceptible to root rot caused by the pathogen. We have completed three cycles of selection in alfalfa for resistance to race 2, resulting in the development of a population that is highly resistant to the disease. We plan to conduct a final cycle of selection for resistance so that alfalfa breeders can use these populations to develop varieties with resistance to the pathogen.

4. Tolerance to Flumioxazin.

Flumioxazin was evaluated for weed control in alfalfa seed and forage production in 2007 and 2008. Applications of flumioxazin to dormant alfalfa in February, on alfalfa planted the previous fall

(either August 15, September 5, or September 26) inhibited early growth and hay yields of the first cutting of alfalfa in 1 of 2 years. Hay yield of the second cutting of alfalfa was not reduced by flumioxazin. Flumioxazin applied in February injured later plantings of alfalfa more than early planting. Established alfalfa treated with flumioxazin in February produced forage and seed yields similar to those obtained by treating with other currently registered herbicides. Data generated have contributed to the registration and new use patterns of flumioxazin in alfalfa seed and forage production.

5. Effect of field burning of alfalfa residue from seed production and alternative residue management practices.

Field burning of alfalfa residues following seed harvest is a common practice in some alfalfa seed production areas and is under scrutiny by clean air advocates. Alternatives to field burning of alfalfa residues are being evaluated in a field experiment on a commercial alfalfa seed production field near Touchet, WA. Alfalfa was seeded at two row spacings (22 and 30 inch) and two seed spacings (1 5/8 and 3 3/8 inch) with a precision seeder in August, 2007. Four residue management treatments were implemented in February 2009 consisting of burning, mowing, shallow tillage, and no residue removal.

6. Weed control and weed seed viability in alfalfa seed production.

Prickly lettuce and Western salsify were the two most prevalent weeds in the field. Field burning destroyed 99% of mayweed and prickly lettuce seed and 95% of salsify seed placed on the soil surface, but had no effect on seed placed 2.5 cm deep. Prickly lettuce density was not affected by the four residue management treatments, whereas Western salsify density was greater where residues were not removed and in mowed treatments. Burning or tilling alfalfa residues reduced Western salsify density by greater than 80%.

7. Managing alfalfa stem rot caused by Sclerotinia sclerotiorum under seed production.

Severity was greater at 22" row spacing with 1 5/8" seed spacing than all other spacings tested. This spacing is the most common spacing currently used by alfalfa growers for seed production and is likely contributing to increased incidence of stem rot. A 30" row spacing with a 3 3/8" seed spacing was determined to have the best combination of yield and low stem rot incidence. It was also determined that row and seed spacing influences the amount of stem tissue that comes in direct contact with the soil. Row and seed spacings that reduce the amount of alfalfa stem tissue in direct contact with soil helps limit stem rot development. There were no significant differences between the row and seed spacing and stubble management treatments on the quantity of sclerotia recovered from the soil. A fungus that feeds on sclerotia has been isolated from several sclerotia and is being investigated as a potential biological control of this pathogen.

8. Weed control and weed seed longevity in forage alfalfa grown under deficit irrigation.

Alfalfa forage production is being studied in alfalfa grown under a line source irrigation system to implement irrigation levels from zero to 100% ET replacement. Within irrigation levels, weed control and weed seed longevity are being evaluated. Hexazinone and flumioxazin controlled most

weeds effectively over all irrigation levels in 2008 and 2009. When no residual herbicide was used, winter annual weed density was greater in both years. In June 2008, wild oat density increased as irrigation level increased. Hexazinone completely controlled wild oats over all irrigation levels whereas, flumioxazin only partially controlled wild oats. Weed seeds (prickly lettuce, pigweed, and salsify) were buried in nylon mesh packets in the spring of 2009 and will be recovered after 6 and 12 months of burial to determine seed viability among different irrigation treatments.

Bio-energy/Feedstocks Production

(Ashok Alva, Rick Boydston, Hal Collins)

1. Production of biochar from animal manure, and its use for cleaning wastewater.

Estimates of animal manures produced in the United States by feedlot cattle, dairy cattle and swine exceeds 78 million tons annually. These manures contain 12.5 million tons of nitrogen (N) and 2.5 million tons of phosphorus (P) in addition to many other important plant nutrients. A typical dairy of 4000 cows produces 29 million gallons of liquid manure a year that must be disposed of in some fashion. The common practice is the application of lagoon water to adjacent agricultural fields. This application can deliver >600 lbs N/acre/year and 160 lbs P/acre/year, exceeding crop needs, resulting in nutrient leaching to groundwater or runoff losses to waterways. We are developing technology to utilize dairy waste as an alternative energy and fertilizer source. The fiber component exiting a GHD™ Plugged Flow anaerobic digester as well as softwood feedstocks was used to produce bio-gas or bio-oil under low temperature pyrolysis. The co-product, biochar was applied to dairy waste water to remove nutrients. Our approach resulted in the removal of 50% of the P from the dairy effluent within 15 days of treatment. Phosphorus concentration of the char following treatment was 20 lbs per ton. Sorption of nutrients by biochar from storage lagoons prior to field application of the effluent will reduce the nutrient loading of soils and contamination of ground and surface waters adjacent to dairies. In addition, the sale of the nutrient enriched biochar off-farm as a fertilizer will increase dairy revenues. Dairies in Washington State alone could produce 230,000 tons of biochar a year from manure.

2. Switchgrass Cultivars Response to Herbicides during Establishment.

Switchgrass has the potential to become a major feedstock for ethanol production. Weeds present during switchgrass establishment reduce switchgrass stands, delay or prevent maximum switchgrass yields, and lower the quality of the switchgrass harvested. Grass weeds are particularly difficult to selectively control during switchgrass establishment and can cause stand failure and the need to replant. Quinclorac and pendimethalin applied in the year of switchgrass establishment controlled both large crabgrass and green foxtail. Mesotrione controlled only large crabgrass during switchgrass establishment. Quinclorac was the only treatment that did not injure switchgrass excessively and has potential for use in managing grass weeds during switchgrass establishment. Identification of effective herbicides for grass weed control during switchgrass establishment provides producers with effective tools to improve success of switchgrass stand establishment, improve switchgrass yields and quality, and increase profitability of switchgrass production.